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ORIGINAL PAPER

The inhibiting effect of nitrate fertilisation on methane uptake of a temperate forest soil is influenced by labile carbon

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Abstract Upland soils are the most important terrestrial sink for the greenhouse gas CH_4. The oxidation of CH_4 is highly influenced by reactive N which is increasingly added to many ecosystems by atmospheric deposition and thereby also alters the labile C pool in the soils. The interacting effects of soil N availability and the labile C pool on CH_4 oxidation are not well understood. We conducted a laboratory experiment with soil columns consisting of homogenised topsoil material from a temperate broad-leaved forest to study the net CH_4 flux under the combined or isolated addition of NO_3^- and glucose as a labile C source. Addition of NO_3^- and glucose reduced the net CH_4 uptake of the soil by 86% and 83%, respectively. The combined addition of both agents led to a nearly complete inhibition of CH_4 uptake (reduction by 99.4%). Our study demonstrates a close link between the availability of C and N and the rate of CH_4 oxidation in temperate forest soils. Continued deposition of NO_3^- has the potential to reduce the sink strength of temperate forest soils for CH_4.

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Introduction

Methane (CH_4) has a 25 times higher global warming potential than carbon dioxide (CO_2). Its present concentration in the atmosphere has more than doubled from 715 to 1774 ppb since pre-industrial times over the past 150 years (Forster et al. 2007). Hence, CH_4 contributes about 15% to the present greenhouse effect of the long-lived greenhouse gases (Forster et al. 2007). Beside chemical oxidation in the troposphere (Crutzen 1991; Denman et al. 2007), biological oxidation in aerobic soils by methanotrophs and nitrifiers represents the second strongest absorber of atmospheric CH_4 (Smith et al. 2000; Le Mer and Roger 2001; Denman et al. 2007). In a recent study, Dutaur and Verchot (2007) calculated the global CH_4 sink of soils to 22.4 Tg $year^{-1}$. The CH_4 uptake of soils in the temperate zone accounts for nearly half of this global sink (10.4 Tg CH_4 $year^{-1}$). It is estimated that temperate forest soils contribute between 3 and 5.7 Tg CH_4 $year^{-1}$ to this sink (Curry 2007; Dutaur and Verchot 2007; Ishizuka et al. 2009). Therefore, any change in the CH_4 sink strength of temperate forest soils as resulting from nitrogen (N) deposition, liming or fertilisation activities, altered forest management or forest conversion is of global interest.

Over the past 200 years, temperate forest ecosystems have already received more than ten times higher anthropogenic N inputs through atmospheric deposition than in pre-industrial times (Holland et al. 1999; Nadelhoffer et al. 1999; Galloway and Cowling 2002; Holland et al. 2005; Magnani et al. 2007). Many studies reported N deposition to be an important factor in the control of CH_4 uptake by forest

soils (Kasimir-Klemedtsson et al. 1997; Butterbach-Bahl et al. 1998). Liu and Greaver (2009) assumed that anthropogenic N addition reduces CH_4 uptake by 3.9 to 9.1 Tg CH_4 $year^{-1}$ at the global scale. These figures compare well with the estimate of Dutaur and Verchot (2007) of a reduction by 17% to 40% of the CH_4 sink strength. Due to the strong impact of N addition on CH_4 uptake, intensive research in the laboratory and the field has addressed this topic (Goldman et al. 1995; Bradford et al. 2001b, 2001a; Jang et al. 2006; Borken and Brumme 2009; Bodelier 2011b). In various field studies (King and Schnell 1994a; Dobbie and Smith 1996; Steinkamp et al. 2001) and laboratory experiments (Adamsen and King 1993; Bender and Conrad 1994; Priemé and Christensen 1997; MacDonald et al. 1997), evidence was found for an inhibiting effect of ammonium (NH_4^+) fertilisation on CH_4 oxidation in forest soils. A reduction of CH_4 oxidation by NH_4^+ addition has been explained by substrate competition between NH_4^+ and CH_4 at the binding sites of the catalysing enzyme CH_4 monooxygenase (MMO) in the first step of the CH_4 oxidation pathway (Bédard and Knowles 1989; Topp and Pattey 1997) resulting in enhanced NH_4^+ oxidation. Other explanations are non-competitive effects exerted by the side-product hydroxylamine during NH_4^+ oxidation (Mancinelli 1995), the production of toxic nitrite (NO_2^-) during NH_4^+ oxidation (King and Schnell 1994b) and osmotic effects resulting from the formation of salts in the course of fertilisation (Dunfield and Knowles 1995; Gulledge and Schimel 1998). Furthermore, several studies reported a negative effect of oxidised N (NO_3^-) on the CH_4 oxidation in soil (Priemé and Christensen 1997; Wang and Ineson 2003; Reay and Nedwell 2004; Xu and Inubushi 2004; Ishizuka et al. 2009). This inhibiting effect has been explained by (a) the production of toxic concentrations of NO_2^- in anaerobic microsites (Adamsen and King 1993; Whalen 2000; Wang and Ineson 2003) or (b) the osmotic effect of salts (Dunfield and Knowles 1995; Gulledge and Schimel 1998). However, the underlying mechanisms of these non-competitive inhibiting effects of oxidised N on CH_4 uptake are not yet sufficiently understood.

There is a recent debate on the influence of N addition on carbon (C) turnover and C stocks in forest soils (Magnani et al. 2007; Dezi et al. 2010; Janssens et al. 2010). According to de Vries et al. (2009), the C sequestration in European forest soils has increased as a consequence of the N deposition in the range of 5 to 23 kg C per kg N added. Thus, it is likely that a continuing high N input into forest soils will further increase the amount of labile C. There is a need to study the consequences of increasing amounts of labile C and N in forest soils and their interaction on CH_4 oxidation which is not well understood. Schnell and King (1995) studied the influences of C compounds as glucose, starch, yeast extract, methanol, ethanol, formate, acetate, malate or lactate on the CH_4 oxidation of incubated forest soil. According to their results, these C compounds neither inhibit nor stimulate CH_4 oxidation. However, the applied concentration may have not been high enough to influence the physiology and activity of the CH_4 oxidising community; moreover, the interaction with N availability was not addressed.

In this study, we focused on the effects of NO_3^- fertilisation in combination with the application of labile C (glucose) on the CH_4 uptake of a deciduous forest soil. We tested the hypotheses that (1) high NO_3^- input leads to a relevant reduction in the CH_4 uptake of the forest soil, (2) glucose neither enhances nor inhibits CH_4 uptake when added alone, but (3) in combination with NO_3^-, it increases the inhibiting effect of NO_3^- on CH_4 uptake.

Materials and methods

Soil characteristics and soil sampling

The soil used for the experiment was sampled in a mixed broad-leaved temperate forest in Hainich National Park, Thuringia, Germany (51°04' N 10°30' E) and was immediately prepared for incubation. At the sampling site, the dominating tree species are *Fagus sylvatica* L., *Fraxinus excelsior* L., *Tilia cordata* Mill., *Tilia platyphyllos* Scop., *Carpinus betulus* L., *Acer pseudoplatanus* L. and *Acer platanoides* L. The soil type is a Stagnic Luvisol (IUSS Working Group WRB 2007) of silty texture containing 1.8% sand, 80.2% silt and 18.1% clay. The sampled soil was free of carbonate (<0.02% of C_{total}), had a pH (KCl) of 3.8 and a base saturation of 22.9%. Material of the upper 0 to 10 cm of the mineral soil was collected, excluding litter material. After collecting the soil material, it was homogenised by passing it through a 5-mm sieve.

Experimental setup

For the main experiment, 16 Plexiglass cylinders (50 cm in height, 17 cm in diameter) were used and each filled with 4 kg of the freshly sieved soil. The water content at the start was 22.7% of the fresh weight. The columns were placed in a random arrangement in the laboratory. A supplementary experiment with 16 additional soil columns of the same dimensions and treated alike was conducted to (1) repeat the findings of the main experiment as well as to (2) exclude possible other effects like proposed inhibitory effects of salt addition and to (3) to have a control without labile C addition. Before the start of the main experiment, we kept the incubated soil for a period of 62 days under laboratory conditions to equilibrate the microbial soil community to the climatic conditions and to balance the gas exchange after

disturbing the natural soil structure. After this pre-experimental phase, the experiment with two treatments (addition of NO_3^- and glucose) lasted for another 62 days with three experimental phases (first, second and third N-fertilisation phase) being distinguished (days 1–20, 21–41 and 42–62, respectively). In the main experiment, the N treatment was replicated eight times. The effect of C addition on CH_4 was interpreted in comparison to the fluxes of the respective N treatment before C addition. At day 0 of the experiment (start of first N-fertilisation phase), day 20 (start of second N-fertilisation phase) and day 41 (start of third N-fertilisation phase), eight randomly chosen soil columns were fertilised with a KNO_3 solution in deionised water with an equivalent of 200 kg N ha^{-1}. The amount of added water was adjusted to reach a water-filled pore space (WFPS) level of 80%. At day 42 (start of third N-fertilisation phase), all 16 soil columns were additionally treated with a glucose solution (equivalent to 9,419 kg C ha^{-1}) to simulate unlimited supply of labile C in the soil (both in the N-fertilised and the untreated control columns). The addition of N and C increased the total N (N_{total}) and organic C (C_{org}) pools in the soil columns in comparison to the initial N_{total} and C_{org} contents by absolute amounts of 7.7% and 8.0% (N) and by 33.8% and 36.0% (C) on day 42 of the experiment in the control and the N-fertilised columns, respectively.

In the supplementary experiment with fourfold replication, we examined the response of CH_4 uptake to the addition of either KNO_3 (NO_3^- source), K_2SO_4 (to test for effects of high K concentrations) or glucose (as a labile C source) using the same amounts of N, K and C (200 kg N ha^{-1}, 552 kg K ha^{-1}, 9,419 kg C ha^{-1}, respectively) and the same soil and cultivation conditions as in the main experiment. Furthermore, the initial soil conditions (apart from a higher C_{org} concentration, Table 2) and all analytical procedures were identical to those in the main experiment. The goal of the supplementary experiment with duration of 21 days was to compare the effects of KNO_3 and K_2SO_4 and to study the effect of a labile C source independent from the NO_3^- effect. This additional experiment also served for measuring the NH_4^+ concentrations in the soil after NO_3^- addition because these data were lost in the main experiment due to technical shortcomings.

The soil columns were installed in a greenhouse with 14 h of low daylight (100 μmol m^{-2} s^{-1} PPFD; OSRAM cool white, Lightcolor 840, Munich, Germany). The water-filled pore space was measured every week by weighing the soil columns. The pore volume and the water-filled pore space were calculated by assuming a particle density of 2.65 g cm^{-3} (Schlichting et al. 1995) and by referring to the measured soil bulk density at the experiment's beginning. The soil temperature of each soil column was measured at a depth of 7.5 cm and a horizontal distance to the column edge of 3 cm using NTC thermistors (Epcos, Munich, Germany) that were logged in 15-min intervals with a CR10 data logger (Campbell Scientific Inc., UT, USA). The soil temperature did not differ between the treatments, but decreased slightly by 3°C over the course of the experiment (mean of 22.8±0.2°C in the N-fertilised and the control treatment).

Chemical soil analysis

Before the start of the experiment, we analysed five replicate samples for the chemical properties of soil (Table 1). The pH (KCl) was analysed in a suspension of 10 g soil in 1 M KCl solution using a Vario pH meter (WTW GmbH, Weilheim, Germany). The cation exchange capacity (CEC) of the soil was measured by percolating five samples of 2 g fresh soil with 0.2 M BaCl. The percolates were analysed with an ICP-AES (Optima 3000 XL, PerkinElmer, MA, USA). The base saturation was calculated as the percentage of base cations (Na, K, Ca and Mg) in CEC. The bulk density of the soil material in the columns was determined using steel cores with a defined volume of 100.93 cm^3. Before and after drying the soil cores at 105°C for 24 h, the soil was weighed, and the bulk density of the dry material was calculated. At day 0 of the fertilisation experiment, the bulk density of the homogenised material was 1.05±0.02 g cm^{-3}. The particle size distribution was determined with the sieving and pipette method (Schlichting et al. 1995). The concentrations of organic C (C_{org}) and total N (N_{total}) were analysed with a C/N analyzer (Vario EL, Elementar, Hanau, Germany). The concentrations of N–NO_3^- and N–NH_4^+ in mg kg^{-1} dw were measured by extracting 8 g fresh soil with 0.5 M K_2SO_4 solution (ratio of wet soil mass to solution, 1:3) within the following 2 to 3 h after collecting the soil. The samples were shaken for 1 h and passed through folded filters (FT-4-303-150, Sartorius Stedim, Aubagne, France). The NO_3^- and NH_4^+ concentrations of the filtered extracts were analysed by continuous flow injection colorimetry (SAN+Continuous Flow Analyzer, Skalar Instruments, Breda, The Netherlands). The NO_3^- concentrations were determined with the copper–cadmium-reduction method (ISO method 13395), and those of NH_4^+ with the Berthelot reaction method (ISO method 11732).

At day 0 and at the end of the experiment, soil solution was sampled from the soil column by irrigating the soil with 500 ml of distilled water. The percolating water was passed through filter papers (MN 85/70 BF, Macherey-Nagel, Düren, Germany) and the soil solution analysed with the DOC analyser (Dimatoc 100, Dimatec, Essen, Germany) to measure the concentration of dissolved organic C. The concentration of NO_3^- in the soil solution was analysed by ion chromatography (761 Compact IC, Metrohm, Herisau, Switzerland).

Table 1 Selected chemical parameters in the soil solution of the columns of the N-fertilised and the unfertilised control treatment in different phases of the experiment (means±1 SE of each eight columns)

Experimental phase	Day 0	Unfertilised control (N0)	N-fertilised (N1)	Unfertilised control (N0)	N-fertilised (N1)
		Day 41 (without glucose)		Day 62 (glucose added)	
pH (KCl)	3.82±0.03	3.84±0.08aA	4.23±0.06bA	4.49±0.07aB	5.63±0.16bB
C_{org} [g kg^{-1} dw]	19.84±0.44	20.44±0.37aA	19.20±0.25bA	22.99±0.58aB	22.62±0.28aB
N_{total} [g kg^{-1} dw]	1.89±0.03	2.08±0.03aA	2.40±0.08bA	1.99±0.01aA	2.42±0.09bA
C/N [g g^{-1}]	10.50±0.19	9.85±0.10aA	8.02±0.17bA	11.52±0.18aB	9.41±0.30bB
NO_3^- [mg Nkg^{-1} dw]	6.39±0.28	ND	ND	0.60±0.80a	35.78±5.70b
NH_4^+ [mg Nkg^{-1} dw]	7.85±0.28	ND	ND	1.53±0.32a	34.50±4.59b
DOC [mg l^{-1}]	19.4±1.91	ND	ND	515.3±89.9a	273.2±51.7b
NO_3^- [mg l^{-1}]	9.6±3.50	ND	ND	30.5±23.6a	363.35±114.7a

Day 0 refers to the start of the experiment; day 41 is 6 weeks after the first N fertilisation and day 62 is 3 weeks after the third N fertilisation combined with glucose addition. Lower case letters indicate significant differences between the N-fertilised and the control treatment within a given experimental phase ($P<0.05$, Wilcoxon *U*-test); different upper case letters indicate significant differences between columns before and after glucose addition ($P<0.05$, Wilcoxon signed rank test)

ND parameter not detected

Gas flux analysis

Gas fluxes of CH_4 at the soil surface were measured three times per week in the headspace volume of soil columns. The headspace volume in the Plexiglass columns was 8.6 L. The chambers were closed for 1 h. At 0, 20, 40 and 60 min elapsed time after lid closure, gas samples were taken from the chamber headspace by flushing gas-tight 50-ml sample syringes with headspace air, using a needle and two three-way valves. The gas concentrations were analysed by a computer-controlled gas chromatographic system with a flame ionization detector for CH_4 (Shimadzu GC-14B, Kyoto, Japan). A detailed description of the gas chromatograph was given by Loftfield et al. (1997). The gas fluxes were calculated from the linear increase of gas concentration, which was measured during the chamber emplacement.

Data analysis

Statistical analyses were performed using SAS 9.1 software (Statistical Analysis System, SAS Institute Inc., Cary, USA). Cumulative gas fluxes were calculated by summing up all measurements for each column considering the number of measurements and the corresponding duration of the measuring phase. Frequency distributions were tested for normality with the Shapiro–Wilk test. One-way GLM with the Tukey–Kramer test was used to identify significant differences among the N-treatment means for cumulative CH_4 fluxes and soil properties showing normal distribution. Not normal-distributed soil parameters were analysed with the Wilcoxon *U*-test. Differences among normal-distributed CH_4 flux data of the different C treatments were assessed with the paired *t* test. The Wilcoxon signed rank test was used to identify differences between the C treatments in not normal-distributed soil parameters (this single test was carried out with the R statistical package, version 2.11.1, R Foundation for Statistical Computing, Vienna, Austria). Linear regression analysis was conducted to relate CH_4 flux to WFPS. For all analyses, significance was determined at $P<0.05$.

Results

Physical and chemical soil characteristics

Important chemical properties of the soil in the columns before the start of the experiment (day 0), at the end of the second N fertilisation phase (day 41) and 3 weeks after the combined application of N and C (day 62) are listed in Table 1. At the end of the second N fertilisation (day 41) and the end of the third experimental phase (combined addition of N and glucose, day 62), the pH (KCl) in the N-fertilised columns was significantly higher (by 0.4 and 1.1 pH units, respectively) than in the unfertilised control. The glucose application strongly increased the pH by 0.7 (control) and 1.4 units (N-fertilised) compared with the corresponding N treatment before glucose was applied. At the 21st and 42nd day of the experiment (with the addition of N or N and C), the WFPS was adjusted to approximately 80% (Fig. 1a). Subsequently, WFPS gradually declined due to soil evaporation with a slope of −1.53±0.22% d^{-1} in the unfertilised soil columns and −1.53±0.24% d^{-1} in the fertilised columns.

The effect of NO_3^-, glucose and K_2SO_4 on C_{org} and the mineral N concentration in the soil was investigated in the supplementary experiment (Table 2). The addition of KNO_3 increased the NO_3^- and NH_4^+ concentrations in the soil

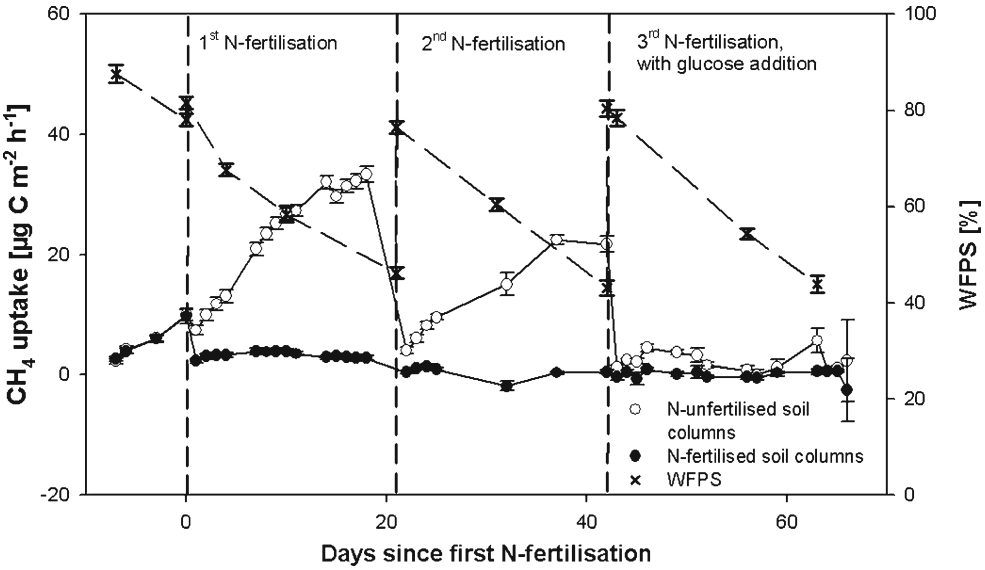

Fig. 1 CH_4 uptake and water-filled pore space of the soil (WFPS) in soil columns containing forest soil, either fertilised with NO_3^- or unfertilised control during the experiment of 62 days duration. Given are mean values±1 SE of each eight columns per N-treatment. The N-fertilization consisted of a total addition of 200 kg N ha^{-1} $year^{-1}$ given as KNO_3 on three occasions (first to third fertilisation). On day 41 of the experiment, all columns received additionally a glucose solution (equivalent to 9,419 kg C ha^{-1}) as a labile C source

columns by 891% and 244%, respectively. K_2SO_4 increased the NO_3^- and NH_4^+ concentrations in the soil by 243% and 134%, respectively. Glucose led to a significant increase of C_{org} and a decline by 81% and 47% of NO_3^- and NH_4^+, respectively.

CH_4 fluxes

During the first phase of the main experiment, the unfertilised control columns showed enhanced uptake of CH_4 with declining WFPS. Maximum uptake (33.31±1.36 µg C m^{-2} h^{-1}) was measured at day 17 when WFPS had dropped to 49.50±1.64%. In contrast, NO_3^- fertilisation reduced CH_4 uptake considerably (Fig. 1). The CH_4 uptake of the fertilised soil remained constantly low at 3.23±0.14 µg C m^{-2} h^{-1} during the first experimental phase. In the second phase of the experiment, the maximum uptake of the unfertilised soil was 22.45± 0.74 µg C m^{-2} h^{-1}. In the N-fertilised soil columns, the second N application led to an even stronger inhibition of CH_4 uptake than during the first phase. The first and second NO_3^- addition significantly reduced the cumulative CH_4 uptake of the N-fertilised soil columns by 86% in the first and by 97% in the second phase compared with the unfertilised columns (Fig. 2).

Glucose application also led to a large reduction in CH_4 uptake with average rates of only 2.41±1.70 µg C m^{-2} h^{-1} during the third phase. The cumulative CH_4 uptake after glucose application was significantly lower in both the unfertilised control and in the N-fertilised columns than in the first and second experimental phases before glucose application (Fig. 2). The glucose addition inhibited CH_4 uptake to a similar extent (by 83%) as did the first NO_3^- addition. After combined NO_3^- and glucose addition in the third phase, the CH_4 uptake was almost completely suppressed (reduction by 99.4% compared with the uptake of the control during the first phase, Fig. 2).

In the unfertilised control treatment of the main experiment, we found a strong negative correlation between CH_4 uptake and WFPS with a large slope factor (higher CH_4 uptake at lower soil moisture) in the first and second phase of the experiment (R^2=0.679 and 0.788, respectively, Fig. 3). After adding NO_3^- in the fertilised treatment, this relationship had a much smaller slope (first phase R^2=0.14),

Table 2 Chemical properties of the soil before (Day 0) and 21 days after the addition of KNO_3, K_2SO_4 or glucose compared with the control treatment in the supplementary experiment (means±1 SE, n=4)

	Day 0	Control	KNO_3	K_2SO_4	Glucose
pH (KCl)	ND	4.32±0.11a	4.15±0.09a	4.12±0.07a	4.20±0.04a
C_{org} [g kg^{-1} dw]	29.20±0.55	29.00±0.23ab	27.61±0.54a	29.00±0.23ab	32.8±1.76 b
N_{total} [g kg^{-1} dw]	2.00±0.02	1.95±0.03a	2.02±0.05a	1.96±0.01a	1.89±0.02a
C/N [g g^{-1}]	14.69±0.32	14.91±0.15a	13.70±0.16a	14.80±0.08a	17.32±0.89b
N–NO_3^- [mg kg^{-1} dw]	3.27±0.61	5.07±3.00ab	45.15±2.44c	12.34±1.07b	0.94±0.81a
N–NH_4^+ [mg kg^{-1} dw]	4.79±0.25	3.96±1.86a	9.65±0.85b	5.29±0.77a	2.10±1.50a

Lower case letters indicate significant differences between the four treatments (P<0.05, Wilcoxon U-test)

ND parameter not detected

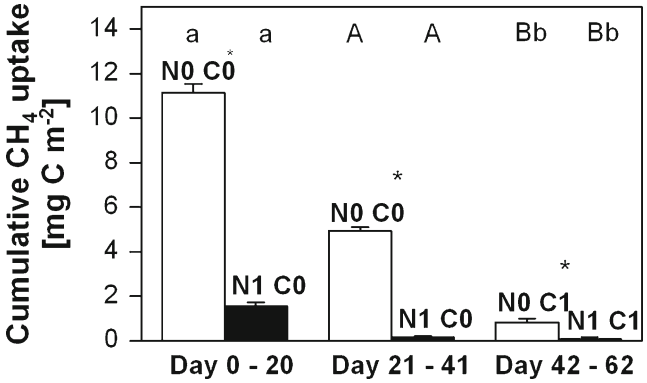

Fig. 2 Calculated cumulative CH_4 uptake in the different treatments during 20 days in the first, second or third phase of the experiment. Treatment acronyms are: *N0*=no NO_3^- fertilisation, *N1*=NO_3^- fertilisation (200 kg N ha^{-1} as KNO_3), *C0*=no glucose addition, *C1*=glucose addition (9,419 kg C ha^{-1}). Given are means±1 SE (n=8 columns per treatment). *Asterisks* mark significant differences between the N-fertilised and the respective control treatment for each phase ($P<0.05$, Tukey–Kramer test), different *lower case letters* indicate significant differences between the first and third experimental phases for the columns of the *C1* and *C0* treatments, and *capital letters* mark such differences between the second and the third phases ($P<0.05$, paired t test)

or disappeared entirely (second phase). After glucose addition, no correlation between CH_4 uptake and WFPS was observed irrespective of the N treatment.

The supplementary experiment with KNO_3, K_2SO_4 or glucose addition showed after 20 days a cumulated CH_4 uptake of only 2.86±0.21 mg C m^{-2} in the KNO_3 treatment, which is equivalent to about half the rate of the control (5.84± 1.2 mg C m^{-2}). In contrast, the columns treated with K_2SO_4 exhibited an enhanced uptake of 7.22±0.18 mg C m^{-2}, which was significantly higher than the control (Fig. 4). The glucose application resulted in the smallest cumulated CH_4 fluxes of the three treatments (−0.04±0.27 mg C m^{-2}, difference significant to the other treatments).

Discussion

Our study showed that KNO_3 relevantly inhibits the CH_4 uptake of a temperate deciduous forest soil. We found a significant reduction of the cumulative CH_4 uptake by 86% after a first addition of KNO_3 equivalent to 200 kg N ha^{-1} (10.4 μmol N g^{-1} dry soil). A second addition of the same amount of N induced a further decline to a rate of only 3% of the control (i.e., a reduction by 97%). The effect of NO_3^- on CH_4 uptake was quite variable in earlier field studies and laboratory experiments. Rigler and Zechmeister-Boltenstern (1999) found a stimulation of the CH_4 uptake of an acidic spruce forest soil after low inputs of 0.71 μmol N g^{-1} dry soil under laboratory conditions (which is a 15th of the 10.4 μmol N g^{-1} dry soil applied in our study). The authors assumed that CH_4 oxidising bacteria may benefit from low N inputs, overcoming N limitation of bacterial growth. In contrast, repeated high inputs of a total of 140 and 530 kg N–NO_3^- ha^{-1} showed no effect on CH_4 uptake of a boreal spruce forest soil under field conditions (Whalen and Reeburgh 2000). Other field and laboratory approaches support our finding of reduced CH_4 uptake as a response to NO_3^- addition (Butterbach-Bahl et al. 1998; Reay and Nedwell 2004; Ishizuka et al. 2009). Nitrate amendments from 0.17 to 29.96 μmol N g^{-1} to soils of temperate mixed hardwood and coniferous forests resulted in a reduction by 10–50% of the initial CH_4 consumption (Wang and Ineson 2003; Xu and Inubushi 2004). A reduction by 86% and 97% measured in our study indicates an even more pronounced inhibition of CH_4 uptake after two consecutive additions of 10.4 μmol N g^{-1} dry soil than reported in earlier studies.

Previous studies suggested that the mechanisms of CH_4 uptake inhibition in the course of NO_3^- addition are linked to (a) substrate competition at the enzyme level and (b) non-competitive effects by the formation of suppressing compounds. High concentrations of NO_2^- formed after the addition of NO_3^- are a well-studied inhibiting factor of microbial activity (Bancroft et al. 1979). Principally, the activity of NO_2^- is relatively high under alkaline pH conditions, at low temperatures or under anaerobic conditions (van Cleemput and Samater 1995). The forest soil used in this study had a low pH value of 4.8, an average temperature of 23°C, a moisture content of less than 80% WFPS and contained an O_2 concentration close to 210 hPa (data not shown; measured with O_2-sensitive micro-optodes, sensor type PSt1 with a resolution between ±0.1 hPa O_2 at concentrations of 2 hPa O_2 and ±0.87 hPa O_2 at concentrations of 207 hPa O_2, with a measurement range between 0 and 500 hPa, Oxy-10 mini and Microx TX3 devices, PreSens GmbH, Regensburg, Germany). Hence, high levels of NO_2^- are not very likely as an inhibiting factor of CH_4 oxidation in our study. Several authors suggested that low osmotic potentials or salt effects caused by high cation concentrations might be another possible non-competitive inhibiting factor associated with N fertilisation (Crill et al. 1994; Hütsch et al. 1994; Bradford et al. 2001a, Bodelier and Laanbroek 2004). A desorption of NH_4^+ from cation exchange sites by high activities of H^+, Na^+ and K^+ cations is one possible mechanism reducing CH_4 oxidation (King and Schnell 1998), while other authors suggested that a lowered soil water potential is responsible for the inhibiting effect of these cations on CH_4 uptake (Nesbit and Breitenbeck 1992; Schnell and King 1996). Wang and Ineson (2003) showed that the effect of K_2SO_4 on CH_4 uptake by a forest soil was only weak (7.0 to 56.1 μmol K g^{-1} dw), whereas the same concentration of KNO_3 strongly depressed consumption rates (7.1 to 56.2 μmol g^{-1} dw of N and K, respectively). In our supplementary experiment, CH_4 uptake remained

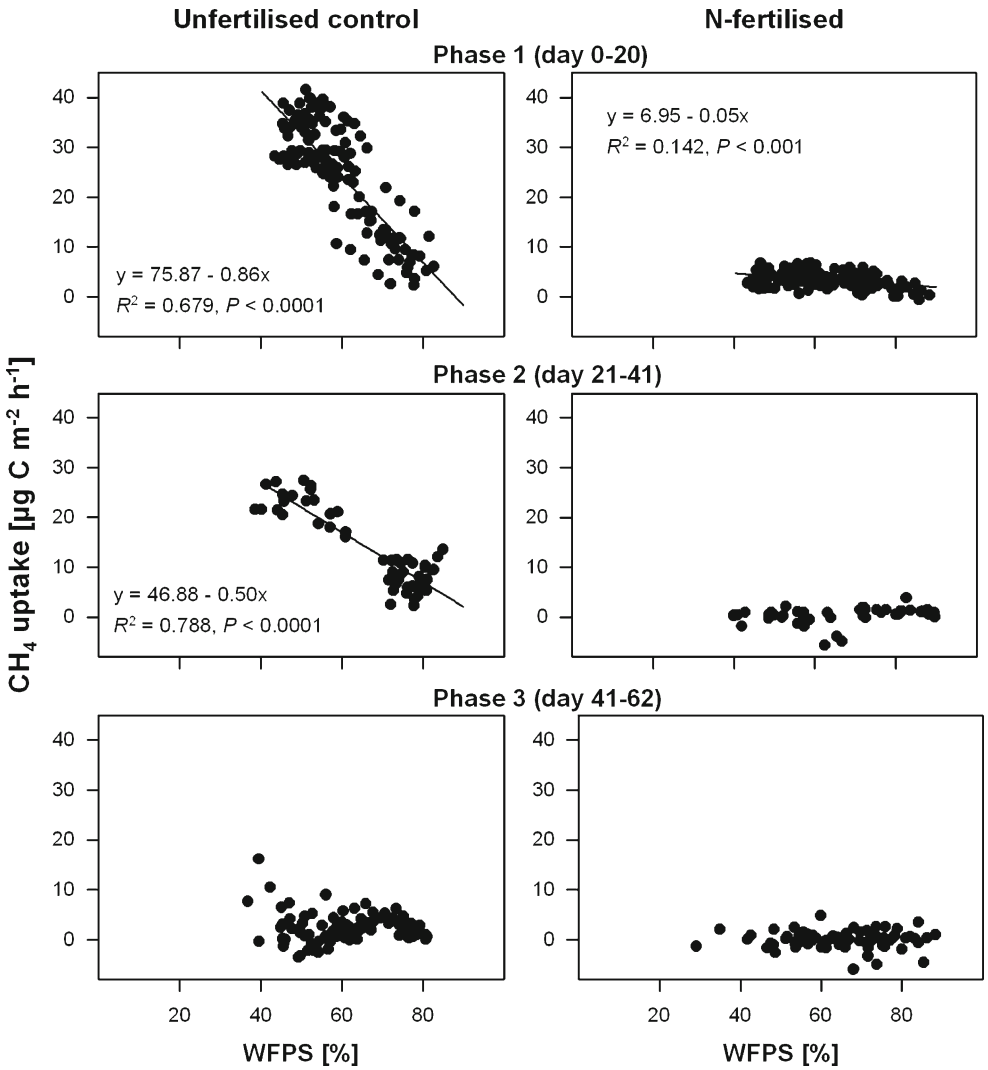

Fig. 3 Dependence of CH_4 uptake rate on the water-filled pore space in the soil (WFPS) in N-fertilised and unfertilised control columns in the first, second and third phase of the experiment (seven up to 14 measurements per phase in each eight columns per treatment). Nitrogen was added as 200 kg N ha^{-1} (KNO_3) at the beginning of the three phases; in the third phase, glucose (9,419 kg C ha^{-1}) was additionally added as a labile C source

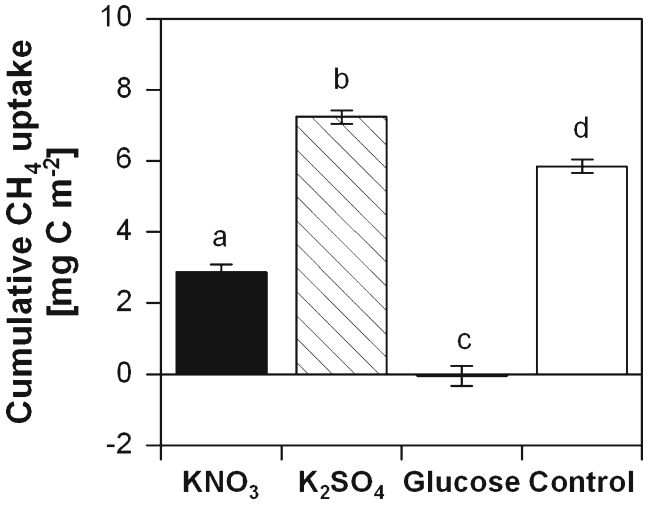

Fig. 4 Calculated cumulative CH_4 uptake after the addition of KNO_3, K_2SO_4 or glucose compared with the control treatment in the supplementary experiment (means±1 SE, n=4). *Lower case letters* indicate significant differences between the four treatments (P<0.05, Tukey–Kramer test)

high after the addition of 10.4 µmol K g^{-1} dw in the form of K_2SO_4, while the addition of KNO_3 with the same amount of K reduced CH_4 uptake by 51%. Thus, a co-determining effect of K on the reduction of CH_4 uptake appears unlikely. The aerobic methanotrophs are a group of methylotrophic bacteria, which are able to use CH_4 and other C1 compounds as their sole energy and C source (Trotsenko and Murrell 2008; Dedysh and Dunfield 2011). Recent studies also showed the existence of facultative methanotrophy in the genera *Methylocystis* (Belova et al. 2011; Im and Semrau 2011), *Methylocapsa* (Dedysh et al. 2005; Dunfield et al. 2010) and *Methylocella* (Dedysh et al. 2005; Theisen et al. 2005). First analysis of the bacterial community within our soil columns derived from the first phase of the experiment shows the presence of the facultative methanotroph genera *Methylocystis*, as well as *Methylocella* and *Methylocapsa*, but in very low abundances (data not shown). We found *Nitrosospira* in the unfertilised soils, but not in the fertilised soil indicating another option of CH_4 oxidation using NH_4^+ monooxygenase (Holmes et al. 1995; Kolb et al. 2005, data

not shown). In our main experiment, the NH_4^+ concentrations increased by 340% compared with the initial concentration after N and C addition and consequently, fertilised columns showed significantly higher NH_4^+ concentrations than the control columns. Similarly, in our supplementary experiment, a single addition of NO_3^- resulted in by 244% higher NH_4^+ concentrations. These increases indicate that the frequently observed inhibition by NH_4^+ must also play a key role for the reduction of CH_4 uptake in our experiments. Why NO_3^- addition led to the strong increase in NH_4^+ concentrations remains unclear. Among the possible mechanisms are a stimulation of N mineralisation by the NO_3^- pulse (Kuzyakov et al. 2000), the assimilation of NO_3^- in bacterial biomass and the subsequent release of NH_4^+ after cell death (Cabello et al. 2009), or perhaps dissimilatory NO_3^- reduction to NH_4^+ in anoxic micro-patches by bacteria.

In general, the WFPS and the O_2 content of the soil are recognised to be the main controlling factors of the temporal variation in CH_4 uptake (Castro et al. 1995; Bowden et al. 1998). Increased soil water content functions as a physical barrier for CH_4 diffusion into the soil, thereby reducing CH_4 uptake (Nesbit and Breitenbeck 1992; Castro et al. 1995). In a field study under natural N supply, Guckland et al. (2009) confirmed the tight negative correlation of CH_4 uptake and WFPS in the soil of the Hainich forest, where the soil material of our experiment was collected. This negative relation was also found in our experiment under the unfertilised control conditions, but it disappeared with NO_3^- addition and its dominant effect on CH_4 oxidation (Fig. 3). Guckland et al. (2009) measured hourly CH_4 uptake rates of 10 to 30 μg C m^{-2} h^{-1} in the National Park Hainich, where our soil material was collected, during midsummer 2007, when soil temperature was 12–14°C and WFPS ranged from 40% to 70%. Although in our study CH_4 uptake of the soil was not investigated under natural conditions, the same range of CH_4 uptake was observed (13–33 μg C m^{-2} h^{-1}, when WFPS ranged from 40% to 70% as well).

Our experiment evidenced not only the strong inhibiting effect of NO_3^- on CH_4 oxidation but also a negative effect by an added labile C compound. With the addition of 576 μmol C g^{-1} dw soil in the form of glucose, the CH_4 uptake of unfertilised soil declined by 83%; adding glucose to NO_3^--fertilised soil caused a reduction by 54% of the initial cumulative CH_4 uptake under NO_3^- fertilisation in the second phase. The repression of CH_4 uptake by the combined action of added N and C was in its absolute amount even larger than the single effects of N and C. Compared with the control of the second experimental phase with no addition of N and C, the cumulative CH_4 uptake of the soil treated with NO_3^- and glucose was reduced by 99.4%, more than the 86% by N and 83% by C addition. To our knowledge, only few studies so far have dealt with the underlying mechanisms of the effect of alternative labile C sources on CH_4 oxidation (i.e., Schnell and King 1995; Benstead et al. 1998). One possible explanation for the inhibition of CH_4 uptake by added labile C sources is the stimulation of heterotrophic microbial processes. We measured an increasing rate of N cycling after the addition of glucose, especially in the treatment with NO_3^- addition where the emission of N_2O was strongly enforced. Nitrate-reducing microorganisms must have been abundant in the soil microbial community in the N1C1 treatment while other processes such as methanotrophy were apparently suppressed. Facultative CH_4 oxidizers are capable of utilising multicarbon compounds, as acetate, succinate, pyruvate, malate or ethanol as their sole C and energy source (Dedysh and Dunfield 2011). Thus, the obvious suppression of CH_4 uptake in the last phase of our experiment could be the consequence of a shift in preference of the methylotrophic bacteria from CH_4 to another multicarbon or C1 substrate, as acetate, pyruvate, ethanol or other side-products of glucose-utilising bacteria. The two enzymes responsible for the oxidation of CH_4 are the particulate and the soluble CH_4 monooxygenase (pMMO and sMMO). The genus *Methylocella* owns only sMMO, which is repressed in the presence of preferred C sources as acetate, malate or other multicarbon substrates (Theisen et al. 2005). In the case of the genera *Methylocapsa* and *Methylacystis*, which prefer CH_4, pMMO and sMMO are present and not repressed in the presence of other C compounds (Dedysh and Dunfield 2011). Finally, we cannot exclude that the apparent low CH_4 uptake rate observed in the N1C1 treatment is partly caused by enhanced CH_4 production because the addition of suitable C substrates may increase methanogenesis under anaerobic conditions (Topp and Pattey 1997; Dalal et al. 2007, Win et al. 2010, Sasada et al. 2011). In fact, the soil moisture conditions in our experiment (40–80 % WFPS) do not exclude the possibility that methanogenesis took place in anaerobic microsites of the not water-saturated soil (Kotiaho et al. 2010). In conclusion, our results suggest that NO_3^- and labile C compounds are agents that may significantly affect CH_4 uptake in temperate forest soils, in addition to the known factors temperature, WFPS and NH_4^+.

For the coming decades, a significant rise in temperatures and in the precipitation extremes is predicted for the temperate zone (IPCC 2007). Another important factor will be atmospheric N deposition, which is expected to remain relatively high in large regions of Central Europe and eastern North America (Galloway et al. 2008). How these expected trends will affect the biogeochemical cycles in forest ecosystems and the chemical state of forest soils is a matter of recent discussion. Much current research focused on alterations of soil C storage and decomposition processes under changed temperature and N immission climates (Janssens et al. 2010), but the interaction between CH_4

uptake and the C and N dynamics in forest soils as schematically described in Dubbs and Whalen (2010) has not received much attention yet (Thornton et al. 2007). The suppression of the CH_4 oxidising community by increasing N may last for decades caused by the narrow and slow-growing community (Bodelier 2011a), so that the CH_4 sink of forest soils might be changed in the long-term. For a number of structurally different forest sites in the Hainich forest, Guckland et al. (2009) reported relatively high CH_4 uptake rates in the range of 2.0 to 3.4 kg C ha^{-1} $year^{-1}$. Guckland et al. (2009) detected no significant correlation between CH_4 uptake and the soil content of C_{org} and NO_3^-. However, N deposition is rather low in the region with 13 kg N ha^{-1} $year^{-1}$ (Mund 2004) and complementary studies in forest stands with higher N input are needed to analyse the interaction between soil C and N availability and CH_4 oxidation under field conditions.

Conclusions

Our study supports the hypothesis of an inhibition of CH_4 uptake by NO_3^- in a biologically active deciduous forest soil. The inhibition by NO_3^- was shown to be linked to increasing NH_4^+ concentrations. The hypothesis of unchanged CH_4 uptake after addition of high amounts of labile C was disproved. In contrast, we found a strong inhibiting influence of a labile C source (glucose) on CH_4 uptake. The simultaneous addition of NO_3^- and a labile C source showed that the inhibiting effects of N and C are more than additive, and the suppression of CH_4 uptake by high soil moisture contents is masked by the dominant N and C influence on this process. These findings underpin the need to investigate the interactions between the availability of NO_3^- and labile C sources on the process of CH_4 oxidation in forest soils. Clearly, our results cannot simply be extrapolated to the field situation because we conducted our experiment with N loads >200 kg ha^{-1}, which is much more than the 10 to 70 kg N ha $year^{-1}$ of N deposition measured currently in temperate European forests (Dise et al. 1998; Holland et al. 2005; Simpson et al. 2006). However, we used artificially high doses of N and labile C to demonstrate that the process of CH_4 uptake in soils is controlled by a number of additional factors that mostly have been ignored so far. Thus, this process is more difficult to predict under changing climatic and chemical conditions in future than previously thought.

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